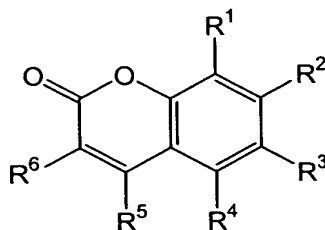


WHAT IS CLAIMED IS:

1 1. A material having a fluorogenic moiety linked to a solid support,
2 said material having the structure:



3
4 wherein:

5 R¹, R², R³, R⁴, R⁵ and R⁶ are members independently selected from the group
6 consisting of H, halogen, -NO₂, -CN, -C(O)_mR⁷, -C(O)NR⁸R⁹, -S(O)_tR¹⁰,
7 -SO₂NR¹¹R¹², -OR¹³, substituted or unsubstituted alkyl, -R¹⁴-SS, and
8 -NHR¹⁵ with the proviso that at least one of R¹, R², R³, R⁴, R⁵ and R⁶ is
9 -R¹⁴-SS and at least one of R¹, R², R³, R⁴, R⁵ and R⁶ is -NHR¹⁵,

10 wherein:

11 R⁷, R⁸, R⁹, R¹⁰, R¹¹, R¹² and R¹³ are members independently
12 selected from the group consisting of H, substituted or
13 unsubstituted alkyl and substituted or unsubstituted aryl;
14 R¹⁴ is a linking group adjoining said fluorogenic moiety and said
15 solid support;

16 R¹⁵ is a member selected from the group consisting of amine
17 protecting groups, -C(O)-AA and -C(O)-P:

18 wherein:

19 P is a peptide sequence;

20 AA is an amino acid residue;

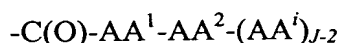
21 m is a member selected from the group consisting of the integers 1
22 and 2;

23 t is a member selected from the group consisting of the integers
24 from 0 to 2; and

25 SS is a solid support.

1 2. The material according to claim 1, wherein said linking group is a
2 member selected from the group consisting of substituted or unsubstituted alkyl,
3 substituted or unsubstituted heteroalkyl and substituted or unsubstituted aryl;

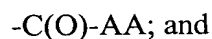
3. The material according to claim 1, wherein P is a peptide sequence comprising the structure:



wherein,

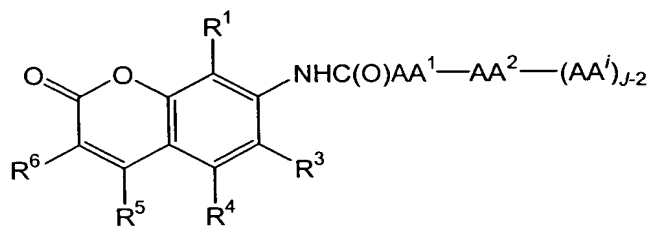
$AA^1-AA^2-(AA^i)_{J-2}$ is a peptide sequence, wherein each of AA^1 through AA^i is an amino acid residue which is a member independently selected from the group of natural amino acid residues, unnatural amino acid residues and modified amino acid residues;
 J denotes the number of amino acid residues forming said peptide sequence and is a member selected from the group consisting of the numbers from 2 to 10, such that $J-2$ is the number of amino acid residues in the peptide sequence exclusive of AA^1-AA^2 ; and
 i denotes the position of said amino acid residue relevant to AA^1 and when J is greater than 2, i is a member selected from the group consisting of the numbers from 3 to 10.

4. The material according to claim 1, wherein R^{15} has the structure:

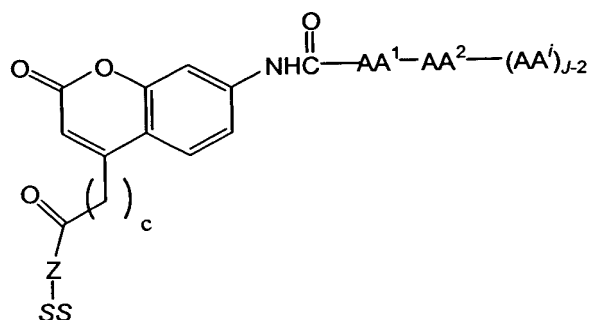


AA is an amino acid residue selected from the group consisting of natural amino acids, unnatural amino acids and modified amino acids.

5. The material according to claim 1, having the structure:



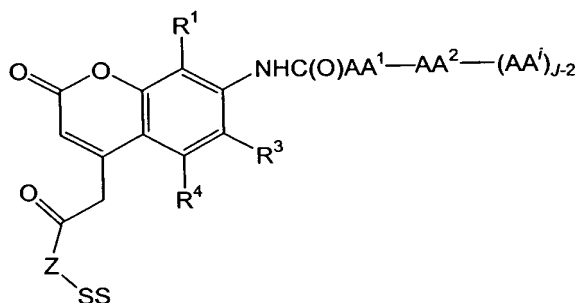
6. The material according to claim 5, having the structure:



wherein, Z is a member selected from the group consisting of -O-, and -NR¹⁶-; and

c is a member selected from the integers from 0 to 6.

7. A material according to claim 6, having the structure:



8. A method of assaying for the presence of an enzymatically active protease in a sample, said method comprising:

(a) contacting said sample with a material according to claim 3 in such a manner whereby said fluorogenic moiety is released from said peptide sequence upon action of said protease, thereby producing a fluorescent moiety; and

(b) observing whether said sample undergoes a detectable change in fluorescence, said detectable change being an indication of the presence of said enzymatically active protease in said sample.

9. The method according to claim 8, wherein said protease is a member selected from the group consisting of aspartic protease, cysteine protease, metalloprotease and serine protease.

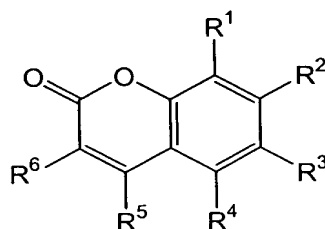
10. The method according to claim 8, wherein said protease is a protease of a microorganism.

each of AA^1 through AA^i is an amino acid residue which is a member independently selected from the group of natural amino acid residues, unnatural amino acid residues and modified amino acid residues;

J denotes the number of amino acid residues forming said peptide sequence and is a member selected from the group consisting of the numbers from 2 to 10, such that $J-2$ is the number of amino acid residues in the peptide sequence exclusive of AA^1-AA^2 ;

i denotes the position of said amino acid residue in sequence relative to AA^1 and when J is greater than 2, i is a member selected from the group consisting of the numbers from 3 to 10; and

R is a fluorogenic moiety having the structure:



wherein:

R^1, R^2, R^3, R^4, R^5 and R^6 are members independently selected from the group consisting of H, halogen, $-NO_2$, $-CN$, $-C(O)_mR^6$, $-C(O)NR^7R^8$, $-S(O)_tR^9$, $-SO_2NR^{10}R^{11}$, $-OR^{12}$, substituted or unsubstituted alkyl, $-NHC(O)-P$, and $-R^{20}-Y$, with the proviso that at least one of R^1, R^2, R^3, R^4, R^5 and R^6 is $-R^{20}-Y$ and at least one of R^1, R^2, R^3, R^4, R^5 and R^6 is $-NHC(O)-P$,

wherein:

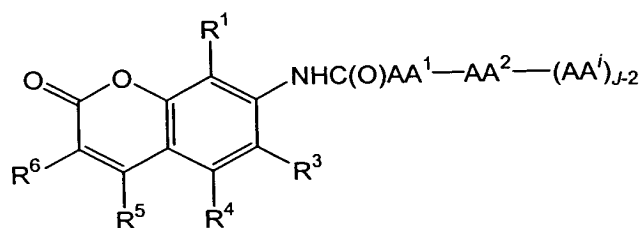
$R^6, R^7, R^8, R^9, R^{10}, R^{11}$ and R^{12} are members independently selected from the group consisting of H, substituted or unsubstituted alkyl and substituted or unsubstituted aryl;

R^{20} is either present or absent and is a member selected from the group consisting of substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl;

37 Y is a member selected from the group consisting of organic
 38 functional groups and methyl;
 39 m is a member selected from the group consisting of the integers 1
 40 and 2; and
 41 t is a member selected from the group consisting of the integers
 42 from 0 to 2.

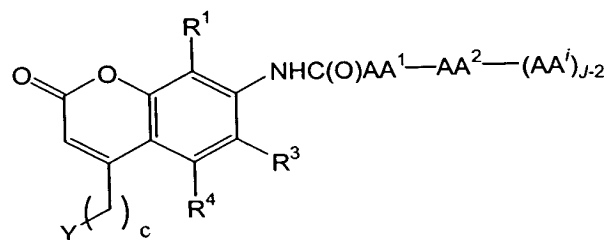
1 17. The fluorogenic peptide according to claim 16, wherein said
 2 organic functional group is a member selected from the group consisting of $-\text{COOR}^{17}$,
 3 $\text{CONR}^{17}\text{R}^{21}$, $-\text{C(O)R}^{17}\text{R}^{21}$, $-\text{OR}^{17}$, $-\text{SR}^{17}$, $-\text{C(O)SR}^{17}$ and $-\text{NR}^{17}\text{R}^{21}$
 4 wherein, R^{17} and R^{21} are members independently selected from H, substituted or
 5 unsubstituted alkyl and substituted or unsubstituted aryl.

1 18. A fluorogenic peptide according to claim 16, having the structure:



2

1 19. A fluorogenic peptide according to claim 18, having the structure:

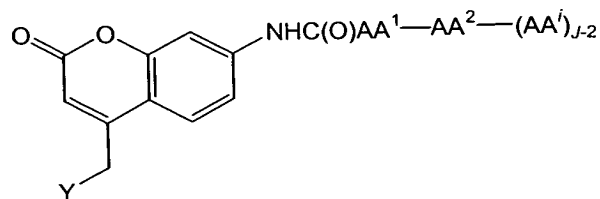


2

3 wherein:

4 c is a member selected from the group consisting of the integers from 0 to 6.

1 20. A fluorogenic peptide according to claim 19, having the structure:



2

1 **21.** The fluorogenic peptide according to claim **16**, wherein said
2 peptide sequence comprises a peptide bond that is cleaved by a protease releasing said
3 fluorogenic moiety from said peptide sequence, thereby producing a fluorescent moiety
4 and a peptide moiety.

1 **22.** The fluorogenic peptide according to claim **21**, wherein said
2 peptide bond is formed between a carboxyl of the carboxy-terminus amino acid residue
3 and an amine group of said fluorogenic moiety.

1 **23.** A method of assaying for the presence of an enzymatically active
2 protease in a sample, said method comprising:

3 (a) contacting a sample suspected of containing said protease with a
4 peptide according to claim **16** in such a manner whereby said fluorogenic moiety is
5 released from said peptide sequence upon action of said protease, thereby producing a
6 fluorescent moiety; and

7 (b) observing whether said sample undergoes a detectable change in
8 fluorescence, said detectable change being an indication of the presence of said
9 enzymatically active protease in said sample.

1 **24.** The method according to claim **23**, wherein said protease is a
2 member selected from the group consisting of aspartic protease, cysteine protease,
3 metalloprotease and serine protease.

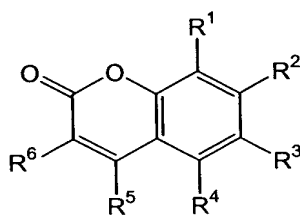
1 **25.** The method according to claim **23**, wherein said protease is a
2 protease of a microorganism.

1 **26.** The method according to claim **25**, wherein said microorganism is
2 a member selected from the group consisting of bacteria, fungi, yeast, viruses, and
3 protozoa.

1 **27.** The method according to claim **23**, wherein said sample is a
2 clinical sample from a subject.

1 **28.** The method according to claim **27**, wherein said subject is a
2 human.

each J is independently selected and denotes the number of amino acid residues forming said first peptide sequence and said second peptide sequence and is a member selected from the group consisting of the numbers from 2 to 10;
each i is independently selected and denotes the position of said amino acid residue relative to AA¹ and when J is greater than 2, i is a member selected from the group consisting of the numbers from 3 to 10; and
for each of said first peptide and said second peptide R is independently selected from fluorogenic moieties having the structure:



wherein:

R^1 , R^2 , R^3 , R^4 , R^5 , and R^6 are members independently selected from the group consisting of H, halogen, $-\text{NO}_2$, $-\text{CN}$, $-\text{C}(\text{O})_m\text{R}^7$, $-\text{C}(\text{O})\text{NR}^8\text{R}^9$, $-\text{S}(\text{O})_t\text{R}^{10}$, $-\text{SO}_2\text{NR}^{11}\text{R}^{12}$, $-\text{OR}^{13}$, substituted or unsubstituted alkyl, $-\text{NH}-\text{C}(\text{O})-\text{P}$, $\text{R}^{20}-\text{Y}$ and $-\text{R}^{14}-\text{SS}$, with the proviso that for each peptide at least one of R^1 , R^2 , R^3 , R^4 and R^5 is a member independently selected from $-\text{R}^{14}-\text{SS}$ and $\text{R}^{20}-\text{Y}$ and at least one of R^1 , R^2 , R^3 , R^4 , R^5 , and R^6 is $-\text{NH}-\text{C}(\text{O})-\text{P}$,

wherein:

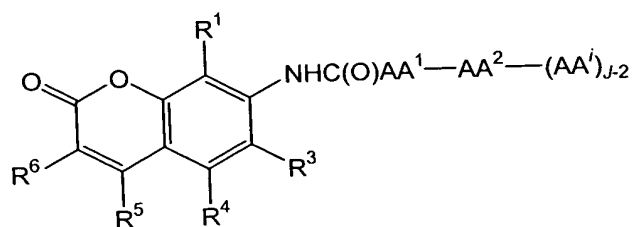
R^7 , R^8 , R^9 , R^{10} , R^{11} , R^{12} and R^{13} are members independently selected from the group consisting of H, substituted or unsubstituted alkyl and substituted or unsubstituted aryl;
 R^{14} is a linking group adjoining said fluorogenic moiety and the solid support;
 R^{20} is either present or absent and is a member selected from the group consisting of substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl;
Y is a member selected from the group consisting of organic functional groups and methyl;

m is a member selected from the group consisting of the integers
 from 1 to 2;
 t is a member selected from the group consisting of the integers
 from 0 to 2;
 Y is a member selected from the group consisting of $-\text{COOR}^{17}$,
 CONHR^{17} , $-\text{C(O)R}^{17}$, $-\text{OR}^{17}$, $-\text{SR}^{17}$, and NHR^{17} ;
 R^{17} is a member selected from the group consisting of H,
 substituted or unsubstituted alkyl and substituted or
 unsubstituted aryl; and
 SS is a solid support.

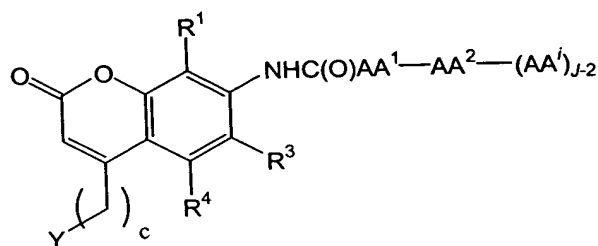
33. The library according to claim 32, wherein said linking group is a
 member selected from the group consisting of substituted or unsubstituted alkyl and
 substituted or unsubstituted heteroalkyl

34. The library according to claim 32, wherein said organic functional
 group is a member selected from the group consisting of $-\text{COOR}^{17}$, $\text{CONR}^{17}\text{R}^{21}$,
 $-\text{C(O)R}^{17}\text{R}^{21}$, $-\text{OR}^{17}$, $-\text{SR}^{17}$, $-\text{C(O)SR}^{17}$, and $-\text{NR}^{17}\text{R}^{21}$
 wherein, R^{17} and R^{21} are members independently selected from H,
 substituted or unsubstituted alkyl and substituted or unsubstituted aryl.

35. The library of fluorogenic peptides according to claim 32, wherein
 R-P has the structure:



36. A library of fluorogenic peptides according to claim 35, wherein
 R-P has the structure:

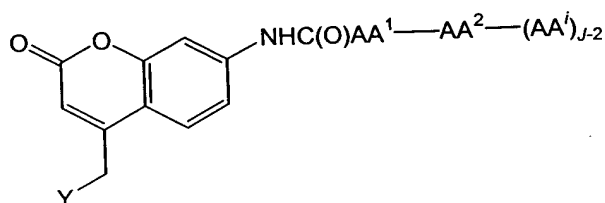


wherein,

c is a member selected from the group consisting of the numbers from 0 to 6.

37. A library of fluorogenic peptides according to claim 36, wherein

R-P has the structure:



38. The library according to claim 32, wherein said fluorogenic moiety of said first peptide and said fluorogenic moiety of said second peptide are different fluorogenic moieties.

39. The library according to claim 32, wherein said first peptide sequence and said second peptide sequence are identical.

40. The library according to claim 32, wherein said first peptide sequence and said second peptide sequence are different.

41. The library according to claim 40, wherein an amino acid residue selected from the group consisting of AA¹, AA², AAⁱ and combinations thereof of said first peptide is a different amino acid residue than an amino acid residue at a corresponding position relative to AA¹ of said second peptide.

42. The library according to claim 32, wherein AA¹ of said first peptide sequence and AA¹ of said second peptide sequence are identical amino acid residues.

1 **43.** The library according to claim **32**, wherein AA¹ of said first
2 peptide sequence and AA¹ of said second peptide sequence are different amino acid
3 residues.

1 **44.** The library according to claim **32**, wherein AA² of said first
2 peptide sequence and AA² of said second peptide sequence are identical amino acid
3 residues.

1 **45.** The library according to claim **32**, wherein AA² of said first
2 peptide sequence and AA² of said second peptide sequence are different amino acid
3 residues.

1 **46.** The library according to claim **32**, wherein AAⁱ of said first peptide
2 sequence and AAⁱ of said second peptide sequence are identical amino acid residues.

1 **47.** The library according to claim **32**, wherein AAⁱ of said first peptide
2 sequence and AAⁱ of said second peptide sequence are different amino acid residues.

1 **48.** The library according to claim **42**, comprising at least six peptides
2 having different peptide sequences, wherein AA¹ is a different amino acid residue in each
3 of said different peptide sequences.

1 **49.** The library according to claim **48**, comprising at least twelve
2 peptides having different peptide sequences wherein AA¹ is a different amino acid residue
3 in each of said different peptide sequences.

1 **50.** The library according to claim **49**, comprising at least twenty
2 peptides having different peptide sequences wherein AA¹ is a different amino acid residue
3 in each of said different peptide sequences.

1 **51.** The library according to claim **32**, wherein AA¹ is a member
2 selected from the group consisting of Lys, Arg, Leu and combinations thereof.

1 **52.** The library according to claim **32**, wherein *J* is a member selected
2 from the numbers from 4 to 8.

1 **53.** The library of peptides according to claim **32**, wherein at least one
2 of said first peptide and said second peptide is cleavable by a protease into a fluorescent
3 moiety and the peptide sequence.

1 **54.** The library according to claim **32**, comprising at least 10 peptides,
2 wherein each of the peptide sequences is a different peptide sequence.

1 **55.** The library according to claim **54**, comprising at least 100 peptides,
2 wherein each of the peptide sequences is a different peptide sequence.

1 **56.** The library according to claim **55**, comprising at least 1,000
2 peptides, wherein each of the peptide sequences is a different peptide sequence.

1 **57.** The library according to claim **56**, comprising at least 10,000
2 peptides, wherein each of the peptide sequences is a different peptide sequence.

1 **58.** The library according to claim **57**, comprising at least 100,000
2 peptides, wherein each of the peptide sequences is a different peptide sequence.

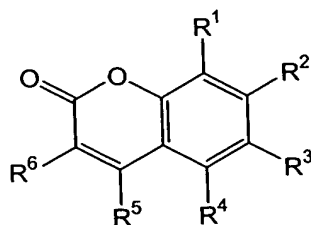
1 **59.** The library according to claim **58** comprising at least 1,000,000
2 peptides, wherein each of the peptide sequences is a different peptide sequence.

1 **60.** The library according to claim **32**, wherein said first peptide is
2 located at a first region of a substrate and said second peptide is located at a second
3 region of a substrate.

1 **61.** A method of determining a peptide sequence specificity profile of
2 an enzymatically active protease, said method comprising:
3 (a) contacting said protease with a library of peptides according to claim
4 **32** in such a manner whereby the fluorogenic moiety is released
5 from the peptide sequence, thereby forming a fluorescent moiety;
6 (b) detecting said fluorescent moiety;
7 (c) determining the sequence of said peptide sequence, thereby
8 determining said peptide sequence specificity profile of said
9 protease.

- 1 **62.** The method according to claim **61**, further comprising (d)
2 quantifying said fluorescent moiety, thereby quantifying said protease.
- 1 **63.** A database comprising at least one set of peptide sequence
2 specificity data for a protease determined using a library according to claim **32**.
- 1 **64.** The database according to claim **63**, wherein said database is an
2 electronic database.
- 1 **65.** The database according to claim **64**, wherein said database is
2 distributed on a wide area network.
- 1 **66.** A database comprising at least one set of peptide sequence
2 specificity data for a protease determined using a method according to claim **61**.
- 1 **67.** The database according to claim **63**, wherein said database is an
2 electronic database.
- 1 **68.** The database according to claim **64**, wherein said database is
2 distributed on a wide area network.
- 1 **69.** The method according to claim **61**, wherein said protease is a
2 member selected from the group consisting of aspartic protease, cysteine protease, and
3 serine protease
- 1 **70.** The method according to claim **61**, wherein said protease is a
2 protease of a microorganism.
- 1 **71.** The method according to claim **70**, wherein said microorganism is
2 a member selected from the group consisting of bacteria, fungi, yeast, viruses, and
3 protozoa.
- 1 **72.** The method according to claim **61**, further comprising (c)
2 quantifying the fluorescent moiety, thereby quantifying said protease.
- 1 **73.** A method of preparing a fluorogenic peptide, said method
2 comprising:

3 (a) providing a first conjugate comprising a fluorogenic moiety covalently bonded
4 to a solid support, said conjugate having the structure:



5
6 wherein,

7 R¹, R², R³, R⁴, R⁵ and R⁶ are members independently selected from the
8 group consisting of H, halogen, -NO₂, -CN,
9 -C(O)_mR⁷, -C(O)NR⁸R⁹, -S(O)_tR¹⁰, -SO₂NR¹¹R¹², -OR¹³ -NR¹⁸R¹⁹,
10 and substituted or unsubstituted alkyl, with the proviso that at least
11 one of R¹, R², R³, R⁴, R⁵ and R⁶ is -NH₂;
12 R⁷, R⁸, R⁹, R¹⁰, R¹¹, R¹², R¹³, R¹⁸ and R¹⁹ are members

13 independently selected from the group consisting of H,
14 substituted or unsubstituted alkyl and substituted or
15 unsubstituted aryl;

16 m is a member selected from the group consisting of the numbers
17 from 1 to 2;

18 t is a member selected from the group consisting of the numbers
19 from 0 to 2;

20 R⁵ and R⁶ are members independently selected from the group consisting
21 of H and -R¹⁴-C(O)NH-SS, wherein at least one of R⁵ and R⁶ is
22 -R¹⁴-C(O)NH-SS;

23 R¹⁴ is a member selected from the group consisting of substituted
24 or unsubstituted alkyl and substituted or unsubstituted
25 heteroalkyl;

26 SS is a solid support;

27 (b) contacting said first conjugate with a first protected amino acid moiety
28 (pAA¹) and an activating agent, thereby forming a peptide bond between a
29 carboxyl group of pAA¹ and the aniline nitrogen of said first conjugate;
30 (c) deprotecting said pAA¹, thereby forming a second conjugate having a reactive
31 AA¹ amine moiety;

32 (d) contacting said second conjugate with a second protected amino acid (pAA²)
33 and an activating agent, thereby forming a peptide bond between a
34 carboxyl group of pAA² and said reactive AA¹ amine moiety; and
35 (e) deprotecting said pAA², thereby forming a third conjugate having a reactive
36 AA² amine moiety.

1 74. The method according to claim 73, further comprising:
2 (f) contacting said third conjugate with a third protected amino acid (pAA³) and
3 an activating agent, thereby forming a peptide bond between a carboxyl
4 group of pAA³ and said reactive AA² amine moiety; and
5 (e) deprotecting said pAA³, thereby forming a fourth conjugate having a reactive
6 AA³ amine moiety.

1 75. The method according to claim 73, further comprising between
2 steps (b) and (c) capping aniline amine groups that have not reacted with pAA¹.

1 76. The method according to claim 75, wherein said capping utilizes a
2 mixture comprising an active ester of a carboxylic acid.

1 77. The method according to claim 78, wherein said active ester is the
2 nitrotriazole ester of acetic acid.

1 78. The method according to claim 74, wherein a member selected
2 from the group consisting of pAA¹, pAA², pAA³ and combinations thereof comprises a
3 mixture of protected amino acids differing in the identity of the amino acid portion of the
4 protected amino acids.

1 79. The method according to claim 78, wherein said mixture comprises
2 at least 2 unique amino acids.

1 80. The method according to claim 79, wherein said mixture comprises
2 at least 6 unique amino acids.

1 81. The method according to claim 80, wherein said mixture comprises
2 at least 12 unique amino acids.

1 **82.** The method according to claim **81**, wherein said mixture comprises
2 at least 20 unique amino acids.

1 **83.** The method according to claim **78**, wherein said mixture is an
2 isokinetic mixture.